REMARKS

Reconsideration and withdrawal of the rejection of the claims, in view of the amendments and remarks herein, is respectfully requested. Claims 3-4 and 35 are amended, and claims 6-7, 17, 21, 23, and 34 are canceled; as a result, claims 1-5, 12-16, 18-20, 22, 27-32, and 35 are now pending in this application.

The Examiner and his supervisor are respectfully thanked for the telephonic interview conducted on October 9, 2007, in which the 35 U.S.C. § 103 rejection was discussed.

Claims 1-7, 12-23, 27-32, and 34-35 were rejected under 35 U.S.C. § 103(a) as being obvious over Schultz-Cherry et al. (U.S. Patent No. 6,696,562), Gulati et al. (J. Clin. Microbiol., 38:4010 (2000)), Black et al. (J. Gen. Virol., 74:143 (1993)), Koci et al. (J. Virol., 74:6173 (2000)) and Kim et al. (WO 92/20803). This rejection is respectfully traversed.

The '562 patent relates to the cloning of turkey astrovirus nucleic acid and the use of RT-PCR to detect that nucleic acid (abstract). It is disclosed that antigenic turkey astrovirus polypeptide fragments may be synthesized in a recombinant system (column 8, line 35-column 9, line 52). Numerous expression systems are disclosed at column 8, line 39-column 9, line 52, i.e., microbial, yeast, insect and mammalian based expression systems. Nevertheless, there is no evidence in the '562 patent that those expression systems were used to prepare any polypeptide. Although a general method of using an antigen to detect antibodies reactive with turkey astrovirus is disclosed at column 14, lines 14-27, the examples in the '562 patent describe the isolation and sequencing of turkey astrovirus sequences, the comparison of those sequences with known sequences, and a RT-PCR assay to detect those sequences.

The '562 patent does not describe a turkey astrovirus recombinant capsid-based assay that specifically detects anti-turkey astrovirus-2 antibodies in animals using capsid-expressing insect cells.

Gulati et al. disclose an avian pneumovirus (APV) matrix (M) protein based assay to detect APV-specific antibodies in turkey sera. The APV protein was expressed as a fusion protein with a His-tag in *E. coli*. The purified fusion protein was coated onto an ELISA plate and sera from control and experimentally APV infected turkeys (28 days after infection) analyzed. The assay detected 97.1% of the experimentally infected birds.

The APV genome has the following structure: 3'-N-P-M-F-M2-SH-G-L-5'. Note that APV N, not APV M, encodes the nucleocapsid protein.

Gulati et al. do not disclose or suggest an assay that specifically detects animals infected with turkey astrovirus-2, e.g., one that employs recombinant turkey astrovirus capsid expressing cells.

Black et al. relate to EIA detection of the antibody response to the M2 protein (an ion channel protein) of influenza virus. The M2 antigen was expressed in insect cells, which were harvested, sonicated, and cell debris removed by centrifugation. The resulting supernatant (soluble M2) was used to coat plates (pages 144-145). The assay detected only 6/17 positive serum samples (P/N ratio $\geq$ 2.0), leading the authors to conclude that the EIA lacked sensitivity (page 145).

The M2 protein of influenza virus is an ion channel protein. Black et al. do not disclose or suggest a viral capsid protein-based assay that specifically detects animals infected with turkey astrovirus-2, e.g., one that employs recombinant protein expressing cells.

Koci et al. disclose the sequence of turkey astrovirus-2 and compare that sequence to other astroviruses.

WO 92/20803 discloses that five serotypes of human astrovirus have been identified (page 5, lines 33-34). The specification discloses methods for the isolation and expression of astrovirus genomic sequences. It is disclosed that astrovirus antigen can be used to identify anti-astrovirus antibodies in serum (pages 22-23), and anti-astrovirus antibodies can be used to detect astrovirus antigens (pages 24-25). It is disclosed that a human astrovirus sequences were isolated from a lambda cDNA expression library using sera from astrovirus-immunized rabbits (Examples 3, 4 and 6). Example 7 discloses how to prepare anti-human astrovirus antibodies.

WO 92/20803 does not describe a viral protein-based assay that specifically detects animals infected with turkey astrovirus-2.

The Examiner is reminded that:

> [i]n order for the Examiner to establish a *prima facie* case of obviousness, three base criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one or ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all

> the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on Applicant's disclosure. M.P.E.P. § 2142 (citing In re Vaeck, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991)).

The primary reference, the '562 patent, describes in detail an actual RT-PCR assay for turkey astrovirus but only generally discloses the detection of antibodies to turkey astrovirus in a sample, i.e., there are no details as to the antigen employed or whether such an assay has an acceptable level of sensitivity. The deficiencies of the '562 patent are not remedied by the remaining cited documents, as Koci et al. describe turkey astrovirus sequences and their relatedness to other astrovirus sequences, WO 92/20803, like the '562 patent, only generally discloses the detection of antibodies to an astrovirus, and the other two cited documents, which disclose serological assays, are unrelated to astroviruses or their detection (Gulati et al. and Black et al.).

Moreover, although the '562 patent and WO 92/20803 (the latter with an effective filing date of 1991) generally disclose methods of using anti-astrovirus antigens, there is no data showing that an assay which employs those antigens is useful to detect antibodies in a blood sample. That is particularly relevant because as late as 2000, Koci et al.(J. of Virol. Methods, 90:79 (2000), "Koci II"; of record) report that detection of astrovirus in most species is limited to electron microscopy and fluorescent antibody detection in cultured (infected) cells but that those methods are time intensive and vulnerable to differences in interpretation (page 79). Notably, Koci II reports a RT-PCR (not a serological assay) to detect turkey astrovirus-2.

Given that Koci II discloses that the detection of astrovirus in most species is limited to electron microscopy and fluorescent antibody detection in cultured cells, the cited art provides no motivation to prepare an astroviral protein-based serological assay, especially in view of the exquisite sensitivity of RT-PCR based assays.

Further evidence of the nonobviousness of Applicant's method is provided in the final Office Action, as the Examiner points out that the '562 patent discloses that "that mammalian cells are good candidates, rather than bacteria or yeast, for producing recombinant antigens since most produce proteins, which mimic the *in vivo* structure of the antigen" (page 4 of the Office Action). While insect cells are also mentioned as possible host cells in the '562 patent, the '562 patent does not suggest that insect cells provide any advantage over the other disclosed host expression systems, e.g., prokaryotic host cells, e.g., *E. coli, Bacillus subtilis, Salmonella, Serratia* and *Pseudomonas*, and eukaryotic host cells, e.g., yeast and mammalian insect cells (column 8, line 39-column 9, line 52).

The Examiner acknowledges that the '562 patent does not teach a specific working example of the above immunoassays involving a fixed insect cell, the presence of IgG or IgM specific for TAstV-2, or a kit for diagnosing a TAstV-2 infection utilizing the above immunoassays," yet concludes that "there would have been a reasonable expectation of success that such an assay would have been able to detect anti-astrovirus-2 capsid antibodies, given the combined teachings of Gulati et al., Black et al., Koci et al. and Kim et al., in view of the '562 patent (overlapping sentence on pages 4-5 of the Office Action).

In this regard, the Examiner is requested to reconsider the Rule 132 Declaration by Dr. Schultz-Cherry submitted on November 30, 2006. In that Declaration, Dr. Schultz-Cherry states that there are number of obstacles in designing a useful serological assay, e.g., recombinant expression of a particular protein may be difficult to achieve, or may not yield sufficient expression levels and/or may not yield a protein that is in a conformation that is recognized by antibodies present in physiological fluid of an animal that has seroconverted. Dr. Schultz-Cherry continues, noting that for a serological assay to be useful, the reagents and conditions must result in sufficient specific binding and low non-specific binding.

The Examiner is also requested to reconsider the Rule 132 Declaration submitted on May 25, 2007, which evidences the obstacles in designing a useful serological assay for turkey astrovirus-2. In that Declaration, Dr. Schultz-Cherry and Dr. Matthew Koci, co-inventors of the present application, state that they tried various systems to express turkey astrovirus-2 capsid protein (paragraph 7 in the Declaration). They found that yields using *in vitro* transcription/translation, transient expression in mammalian cell lines and expression in bacterial cells were generally poor and/or variable (paragraph 7 in the Declaration). Dr. Schultz-Cherry and Dr. Matthew Koci state that of the tested systems, the *in vitro* transcription/translation system produced the best results and was initially chosen for developing the serological assay (paragraph 7 in the Declaration). However, issues related to consistent antigen preparation led to concerns in the reproducibility of assays that employed capsid produced by *in vitro* transcription/translation to detect anti-turkey astrovirus-2 antibodies in serum (paragraph 7 in the Declaration).

An additional obstacle in developing a serological assay for turkey astrovirus-2 related to the amount, and/or affinity for turkey astrovirus-2 capsid, of anti-turkey astrovirus-2 antibodies found in physiological samples (paragraphs 6, 8 and 9 in the Declaration). Dr. Schultz-Cherry and Dr. Matthew Koci disclose that of the antigens tested, only turkey astrovirus-2 capsid expressed in insect cells reproducibly and specifically detected anti-turkey astrovirus-2 antibodies in serum.

The Examiner asserts that the Rule 132 Declaration (filed on May 25, 2007) is insufficient to overcome the rejection, as Gulati et al. was intended to rebut the argument that cholesterols impact serum screening for antibodies and Black et al. allegedly addressed the requirement that the capsid antigen be expressed in insect cells that are fixed or lysates of such cells for use in the claimed method.

The method in Gulati et al., in contrast to Applicant's method, employed a purified fusion protein of a His tag and the APV M protein prepared from *E. coli* to coat an ELISA plate (see page 4011). It is disclosed in Gulati et al. that results obtained from screening serum samples with the purified fusion were superior to those obtained with clarified cell lysates prepared from APV infected Vero cells ("routine APV ELISA") (i.e., the purified M fusion protein ELISA found that 72.3% of the samples were positive while the routine APV ELISA showed that 53.8% of the samples were positive). Thus, Gulati et al. teach away from cell-based lysates as an antigen to detect antibodies, as those assays were less sensitive.

With regard to Black et al., four different assays are disclosed. For one assay, it is disclosed that insect cells infected with a baculovirus that expressed influenza virus M2 protein were fixed, and then contacted with a murine monoclonal anti-M2 antibody followed by a labeled anti-mouse antibody (IFA, Figure 1). In a second assay, infected insect cells were treated with detergent and subjected to Western blot analysis with the monoclonal antibody (see Figure 2). In a third assay, lysates of infected insect cells were subjected to Western blot analysis with experimentally infected ferret serum. Black et al. also disclose an assay in which purified M2 antigen from infected insect cells (see overlapping paragraph at pages 144-145, where it is disclosed that infected cells were pelleted, treated with an alkaline solution, sonicated, subjected to centrifugation, after which supernatents were coated onto plates) was employed in an EIA with human serum. It is disclosed that the EIA assay lacked sensitivity relative to Western blot (which employed detergent treated cell lysates) (see page 145).

Thus, both Gulati et al. and Black et al. employed purified protein and cell lysates to detect antibodies in a physiological fluid sample. Gulati et al. teach that the purified protein is better than the lysate to detect anti-APV antibodies in serum, while Black et al. teach that Western blot with cell lysates was more sensitive than an EIA that employed a purified protein to detect anti-influenza virus antibodies in serum.

Thus, Black et al. provide further evidence of the unpredictability of the sensitivity of serological based assays that employ antigens expressed in insect cells.

Accordingly, the cited art provides no reasonable expectation that recombinant insect cells or lysates thereof are useful to detect antibodies in blood-based samples. Moreover, the differential results in Gulati et al. and Black et al. may, at least in part, be due to the cholesterol in avian serum. **(TRUE?)** For example, cholesterol is less likely to interfere with serological assays that employ "purified" antigen, e.g., purified fusion proteins or proteins separated by electrophoresis and transferred to a nylon membrane.

The Examiner appears to employ impermissible hindsight to arrive at Applicant's invention, picking detection of turkey astrovirus-2, rather than human astrovirus, influenza virus or avian pneumovirus, choosing a serological rather than an antibody (capture) based assay or a nucleic acid-based assay, and choosing recombinant viral protein-expressing cells rather than purified recombinant *E. coli* antigens or solubilized recombinant antigens from insect cells. Nevertheless, as discussed above, there is no combination of the cited documents that discloses, suggests, provides the motivation for, or provides the reasonable expectation of success of, the use of insect cells, e.g., fixed insect cells, expressing turkey astrovirus-2 capsid protein in a serological assay.

Therefore, withdrawal of the § 103 rejection is respectfully requested.

CONCLUSION

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (612) 373-6959 to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

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Date October 31, 2007 By
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CERTIFICATE UNDER 37 CFR § 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Mail Stop RCE, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on this 31st day of October 2007.

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